Fiscal Year:	FY 2017	**Task Last Updated:**	FY 08/03/2016
PI Name:	Lorenzi, Hernan Ph.D.		
Project Title:	Study of the Impact of Long-term Space Travel on the Astronaut's Microbiome		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
Human Research Program Risks:	(1) **Medical Conditions**:Risk of Adverse Health Outcomes and Decrements in Performance Due to Medical Conditions that occur in Mission, as well as Long Term Health Outcomes Due to Mission Exposures (2) **Microhost**:Risk of Adverse Health Effects Due to Host-Microorganism Interactions		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	20892	**Congressional District:**	8
Comments:			
Project Type:	Flight	**Solicitation / Funding Source:**	2010 Crew Health NNJ10ZSA003N
Start Date:	10/01/2011	**End Date:**	09/30/2017
No. of Post Docs:	1	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA ARC
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Flight Program:	ISS		
Flight Assignment:	ISS NOTE: Element change to Human Health Countermeasures; previously Space Human Factors & Habitability (Ed., 1/18/17) NOTE: Extended to 9/30/2017 per F. Hernandez/ARC (Ed., 3/11/16) NOTE: Extended to 9/30/2016 per A. Chu/ARC (Ed., 8/5/14) NOTE: Gap changes per IRP Rev E (Ed., 3/19/14)		
Key Personnel Changes/Previous PI:	Drs. Mark Ott and Duane Pierson are collaborators on this project. March 2016: Added Research Assistant Kelvin Moncera as key personnel to the study. February 2015: Added Key personnel Postdoctoral fellow Alexander Voorhies and Dr. Karen Nelson to the study. July 2014: Manolito Torralba and Dr. Satish Mehta have been incorporated as key personnel. August 2012: Scott Peterson (former co-PI of the project) and Shannon Williamson (key personnel) are not participating in this project any more.		
COI Name (Institution):	Pierson, Duane (Johnson Space Center) Ott, Charlie (Johnson Space Center)		

Grant/Contract No.:	NNX12AB02G
Performance Goal No.:	
Performance Goal Text:	
Task Description:	Our goal is to determine how the composition of the human microbiome changes during long-term space exploration and to evaluate its potential impact on astronauts' health. Some microbial species from the human microbiome have a beneficial or protective effect on health; the loss of these species can lead to an altered metabolic function and, in conjunction with reduced immune response, may increase the chance of infection by opportunistic pathogens. In our proposal we will elaborate the notion of the microbiome as harbingers or sentinels to monitor a variety of aspects of the human host, including associations with health status, environmental stress, and exposure to space conditions. By sampling the microbiome of astronauts on Earth while in peak physical health and during subsequent times of stress, including long-term exposure to microgravity, g-forces, radiation, and changes in health status, we will be able to define signatures of human response to a variety of relevant aspects of space travel. We propose to characterize the bacterial and viral microbiome from various body sites of up to nine astronauts who travel to space at several time points before, during, and after a space mission. Also we will assess the astronauts' immune function before, during, and after the mission by analyzing their collected saliva samples for reactivated latent viruses and cortisol levels, two indicators commonly evaluated during spaceflight immune and stress studies and cytokines from blood samples. Finally, we will correlate the collected microbiome and immune function data with other measured metadata including astronaut health and hygiene as well as environmental factors such as temperature, humidity, and environmental microbial samples that will be collected, depending upon availability, from various surfaces on the International Space Station (ISS).
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The results of this study will provide insights into how the microbial population of the environment affects the composition and dynamics of the human microbiome. This is relevant to studies of respiratory diseases such as asthma and allergies. Investigating the impact of stress and status of the immune system on the human microbiome, and potentially on human health, during a space mission is also applicable to equivalent stressful situations on Earth. Some of the conclusions of this project will also be useful in situations where a group of individuals are confined in a relatively small and closed space for a long period of time, such as a submarine crew.
	1- Inform Consent Briefings, Recruitment of Astronauts, and Base Data Collection We have recruited all 9 subjects requested for the study, named A to I, plus one backup volunteer, and inform consents have been already signed by all of them. Almost all pre-flight, in-flight and post-flight sample sets have been already collected and are at different degrees of processing, except for one set of blood and saliva samples from astronaut F. Swab, stool, and water samples were delivered to J Craig Venter Institute (JCVI) for 16S and metagenomic sequencing and analysis. Saliva and Blood samples were sent to Johnson Space Center (JSC) for measurement of cytokines (immune response), and virus reactivation and cortisol levels (to evaluate astronauts' stress levels) in blood and saliva samples, respectively. Sample sets from each astronaut are being processed altogether to reduce variation due to methodological error. 2- Sequencing and preliminary analysis of 16S rRNA gene taxonomic profiles. A variable region from the bacterial 16S rRNA gene was amplified by total DNA extracted from 399 participant swab samples (75 forehead, 75 forearm, 75 nares, 75 tongue, and 75 negative controls), 63 fecal samples, and 63 International Space Station (ISS) environmental samples (60 from different surfaces and 3 from the water tank) and sequenced in batches of 250 samples per Illumina MiSeq run. The last batch of 140 samples, received on June 22 2016, is currently being amplified by PCR for sequencing. Of the 399 samples sequenced, 156 were processed through an in-house 16S sequence analysis pipeline for a preliminary analysis of the 16S data. These preliminary results, mostly derived from astronauts C and H, indicated that the gastrointestinal (GI) tract microbiota of astronaut C changed during space flight, showing an increase in alpha diversity, mostly explained by a change in the relative abundance of bacterial species and not due to a gain or lost of species. This result was further confirmed by Unifrac analysis. The alteration in relative abundance seems to revert to its original pre-flight state 30 days after the return from space. Similar trends were observed for the skin, nose, and tongue microbiomes of astronauts C and H, although for some sites the microbiomes did not revert to their original composition, once the astronaut returned from space. Interestingly, we found at least one case of bacteria, genus Acidocella, that were abundant in both in-flight and post-flight skin samples from astronauts H, E, and C but absent or at very low abundance in pre-flight samples. This genus is not commonly found in human skin but was found in significant quantities in the ISS environmental samples. This strongly suggests that the exposure of astronauts to the ISS environment may lead to a long-term acquisition of environmental bacteria that are not usually part of the human microbiome, and that the acquired bacteria may persist for long periods of time in the microbiome after the astronauts return to Earth. Comparisons between the astronauts' and the ISS microbiota also revealed an association between the skin and the ISS microbial communities. The only exception was the bacteria collected from the ISS Intermodular Ventilation Inlet (IMV) that resembled the astronauts' nose microbiome. Because the clustering of 16S sequencing data into Operational Taxonomic Units (OTUs) is affected by the initial collection of sequencing reads, it is not recommended to compare taxonomic profiles generated from two different batches of sequences that were processed independently from each other through the 16S sequence analysis pipeline. Therefore, we will proceed with a second (and final) round of taxonomic profiling, which will include all the microbiome samples collected for the project, once the last batch of samples is sequenced at JCVI (expected by the end of August 2016). 3- Metagenomic sequencing of astronauts' microbiome isolates. Although the sequencing and posterior comparative analysis of 16S-based taxonomic profiles allow the characterization

Task Progress:	of compositional changes of the crew microbiome, this type of analysis does not provide in-depth information about how alterations in commensal bacteria may affect their functional interaction with the human host. To fill this gap, we are carrying out a metagenomic sequencing approach on microbial samples collected from the GI (gastrointestinal) tract, nose, and tongue of up to five astronauts, totaling 123 microbiome samples. Currently all the samples have been sequenced with Illumina NextSeq technology using 150 bp paired-end reads at a sequencing depth of approximately 1 Gbp of sequencing data per sample (~164 Gbp total). Metagenomic sequencing reads derived from each astronaut were assembled into contigs with SPAdes, followed by bacterial gene identification. Gene sequences were then processed through a JCVI functional annotation pipeline to predict gene functions and metabolic pathways likely to be encoded by each of the astronauts' assembled metagenomes. Because sequencing reads from nose microbiomes are underrepresented in the pool of microbial reads generated from each astronaut, the resulting metagenome assemblies contained a low amount of contigs representing nose microbiomes. To tackle this issue, we performed a nares-specific assembly by pooling and assembling all the nose sequencing reads generated from the five astronauts. Predicted gene functions were used to identify biological features, known as genome properties, that were likely to be encoded by each of the assembled metagenomes. Genome properties include functional features of interest such as virulence factors, metabolic pathways, and biofilm formation, encoded by microbial genes, whose relative abundance may change during a space mission. Therefore, this type of analysis may shed light into how changes in the human microbiota associated with space travel may affect crew health. To investigate how genome properties encoded by each metagenome vary throughout a mission, microbial metagenomic sequencing reads from each sample were mapped onto their corresponding assemblies with the program BWA and mapping information was then used to quantify how the relative abundance of individual microbial genes changed during the mission. Mapping of all reads is already completed and we are currently in the process of performing comparative analysis of the microbial gene frequencies to identify those genome properties that are affected by space travel. 4- Changes in cytokine concentration in plasma Changes in the level of different cytokines in plasma samples collected at five time points during the mission (L-180, FD10, R-1, R+0, and R+180) are being measured in order to assess the immune response of participating astronauts and to investigate its potential association with taxonomic or functional changes in the crew microbiomes. Thirty-nine out of the 45 plasma samples to be collected in this study have been already processed and cytokine measurements completed. Four out of the five remaining samples (all from astronaut F) have been collected and will be processed once the last sample from astronaut F is delivered to the JSC to avoid batch effects that may arise from technical sources of variation. Comparative analysis of cytokine profiles from the eight completed set of samples using Principal Component Analysis (PCA) revealed that the cytokine profiles from samples collected after 180 days in space (FD180 or R-1) are significantly different from those of pre-flight samples (Time point L-180). Further analysis of changes in the plasma concentration of individual cytokines at different time points during the mission, compared with the initial pre-flight concentration at L-180, revealed similar findings as those described previously in another study, carried out by Crucian et al. in 2014, for ISS astronauts during spaceflight. In agreement with that study, we detected significant in-flight increases in the C-C motif chemokine ligand 2 (CCL2, p=0.01) and the anti-inflammatory interleukin 1-ra (IL-1ra, p=0.003) as well as trends toward in-flight increases for two inflammatory cytokines, tumor necrosis factor alpha (TNFa, p=0.03) and interleukin 8 (IL-8, p=0.03). All these cytokines participate in the inflammatory response. Unexpectedly, our analysis also identified a significant in-flight increase in interleukin 2 (IL-2, p=0.007) that was not seen before in the study by Crucian et al. Interleukin 2 participates in adaptive immunity, primarily by promoting the differentiation of T cells. Also, some changes associated with spaceflight reported previously (Tpo, VEGF) were not observed in this study. This discordance between the two investigations may be due to differences in sample handling and processing. 5- Detection of viral DNA and cortisol levels in saliva samples Processing of saliva samples is still ongoing. As reported in 2015, measurement of cortisol levels and virus reactivation in saliva samples are already done for four astronauts, C, E, G, and H, and we are currently processing four additional complete sets of saliva samples from astronauts A, B, D, and I. As with the cytokine samples, it is recommended to do these measurements once all the samples from a particular astronaut are collected and available for processing to reduce methodological errors and batch effects. Processing of samples from astronaut F will start once the last set of saliva samples are collected for time point R+180.
Bibliography Type:	Description: (Last Updated: 04/10/2021)
Abstracts for Journals and Proceedings	Voorhies AA, Ott CM, Mehta SK, Torralba M, Pierson DL, Lorenzi HA. "Study of the impact of long-term space travel on the astronauts' microbiome." Presented at the 2016 NASA Human Research Program Investigators' Workshop, Galveston TX, Feb 8-11, 2016. 2016 NASA Human Research Program Investigators' Workshop, Galveston TX, Feb 8-11, 2016. , Feb-2016
Abstracts for Journals and Proceedings	Voorhies AA, Ott CM, Mehta SK, Torralba M, Pierson DL, Lorenzi HA. "Study of the impact of long-term space travel on the astronauts' microbiome." Presentad at the 31st Annual Meeting of the American Society for Gravitational and Space Research, Alexandria, VA, November 11-14, 2015. 31st Annual Meeting of the American Society for Gravitational and Space Research, Alexandria, VA, November 11-14, 2015. , Nov-2015
Articles in Peer-reviewed Journals	Voorhies AA, Lorenzi HA. "The challenge of maintaining a healthy microbiome during long-duration space missions." Frontiers in Astronomy and Space Sciences. 2016 July 22 available online. http://dx.doi.org/10.3389/fspas.2016.00023 , Jul-2016